

NOTES ON THE TREMATODE GENUS CLINOSTOMUM

BY WILLIAM WALTER CORT*

During the fall of 1911 I found encysted in the mesenteries and under the peritoneum of several specimens of the leopard frog (*Rana pipiens*) a number of larval distomes belonging to the genus *Clinostomum* Leidy. Recently more cysts were found in a frog of the same species from North Judson, Indiana. Two specimens of this genus from cysts in the black bass (*Micropterus* sp.) near Washington, D. C., were sent me by Dr. B. H. Ransom, and some of the same kind of material was given me by Dr. George R. La Rue, which he had found in perch (*Perca flavescens*) from Douglas Lake, Michigan. Additional material of the larval stages of this genus was turned over to me by Professor Henry B. Ward from his private collection. This material was from the following hosts and localities: From cysts in frog, Oshkosh, Wisconsin; from perch (*Perca flavescens*) and blue gill (*Lepomis pallidus*), Bass Lake, Michigan; from rock bass (*Ambloplites* sp.), Alma, Michigan; from perch, Lake Spooner, Wisconsin; from black bass (*Micropterus* sp.), Sebago Lake, Maine. Also two adults were collected from the mouth of a black-crowned night heron (*Nycticorax nycticorax naevius*) which was taken alive near Urbana, Illinois, and given me by Professor Frank Smith. e/

I wish to express my appreciation of the helpful suggestions and criticisms given me by Professor Henry B. Ward during the preparation of this paper.

During the past year I have been engaged in a comparative study of the above material. Two recent papers by Osborn on the North American representatives of the genus *Clinostomum* have covered much the same ground as my studies. The first of these (Osborn, 1911), considers the distribution and behavior of this type of trematodes, and goes over the literature thoroughly. The second

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(Osborn, 1912), takes up their anatomy in so much detail that further morphological description seems unnecessary at the present time. Although in the main my observations confirm Osborn's, I must dissent from his conclusions in regard to the forms from the frog. Osborn finds so little difference in form and proportions of the body between the late immature stages from cysts in the fish and frog and the mature worms from the heron, that he considers all the flukes of this genus so far collected from North America to belong to the same species, *Clinostomum marginatum* Rudolphi. I find that the specimens from the frog show so many differences that I am forced to consider them as a distinct species. The *Clinostomum* from the bittern (Wright, 1879) also shows several important points of difference from the adults from the heron, and is probably the adult stage of the frog species.

Distomum reticulatum described by Looss (1885), a larval *Clinostomum* from Costa Rica (not Porto Rica, as stated by Osborn and Linton), has given rise to considerable discussion. This form is much larger than any of those from North America, has a lobed ovary, and the cirrus sac extends to a point posterior to the posterior testis. Osborn (1912: 219-220) doubts whether these differences are great enough for specific distinction, and states that too much importance should not be attached to differences in shape of an organ like the uterus or its parts. He further states that writers from Leuckart down have considered *Distomum reticulatum* Looss to be identical with the North American representatives of the genus *Clinostomum*. This statement is not correct since Braun (1900: 44-45) states that *Distomum reticulatum* is distinct from the North American species, and relates the former closely to *Clinostomum sorbens*, a South American species which he described. I agree with Braun's conclusion. Since Looss' name for this form was preoccupied Monticelli renamed it *Mesogonimus dictyotus*. But he overlooked Leidy's earlier genus *Clinostomum*; therefore Looss' Costa Rican trematode should be known as *Clinostomum dictyotum*.

In the study of the North American representatives of *Clinostomum* very little attention has been given to those from frog hosts. MacCallum (1899) reports them, and Osborn (1911) describes their position in the cyst. He also includes them in his later paper

on the structure of *Clinostomum marginatum*, although apparently all his anatomical descriptions and figures represent specimens from the black bass and heron. Both the above authors have referred this form to *Clinostomum marginatum*. I propose to show that the *Clinostomum* form from the frog belongs to a distinct species, which may be named *Clinostomum attenuatum*, since it is the most slender of all the species so far described in this genus.

The late larval stages of *Clinostomum attenuatum* have been found encysted only in frogs, and of *Clinostomum marginatum* only in fish. The cysts in the frogs are scattered in the mesenteries, embedded under the peritoneum of the body cavity and in the lymph spaces between the skin and muscles of various parts. They are never found within the muscle tissue surrounded by its fibers. The cysts from the fish in my experience and Osborn's are always found in the midst of muscle bundles.

Osborn (1911) notes the position of the worm in the cyst as the only difference between the forms from the fish and frog. I have found this condition to be dependent upon the location of the cyst. In those cysts embedded in the muscles of the fish where there is considerable pressure from all sides, the worm is folded three times with the acetabulum to the outside and is very tightly enclosed by its cyst. Where the cysts are loosely held in the mesenteries of the frog and subjected to little if any external pressure, the worm is very loosely enclosed in its cyst, and is usually folded but once. But in those cysts which are located in the frog where they are subjected to pressure as between the large muscles of the upper thigh, the worm is folded three times and compressed tightly in its cyst, offering then much the same appearance as the fish cysts.

Differences are to be noted between the two species in size and shape. In length the range of variation within each species is so great that no constant difference is found. The specimens from the frog ranged from 3.9 mm. to 5.52 mm. in length. Among those from the fish the smallest individual measured 3.5 mm. and the largest 6.6 mm. in length. In body shape the differences are very distinct and constant. The frog type is slender and of almost uniform width and thickness throughout its length (Fig. 1), while the fish type has a broad rather flat post-acetabular region (Fig. 2). In *Clinosto-*

mum attentuatum the pre-acetabular region is oval, measuring on the average about 0.67 mm. in width by 0.41 mm. in thickness, while the whole body back of the acetabulum has a thickness greater than half its width. The average measurement for the region of the ovary are about 0.68 mm. in width by 0.37 mm. in thickness. In *Clinostomum marginatum* from the fish cysts the pre-acetabular region is more nearly cylindrical, the average width for my specimens being 1.05 mm. and the average thickness 0.85 mm. Just back of the acetabulum the ratio of the width to the thickness is about three to two, the average width being 1.35 mm. and the average thickness 0.91 mm. At the ovary, however, this worm is wider and more flattened than the other species, having an average width of 1.55 mm. and a thickness of 0.71 mm. The difference between these two species in relative width of the different body regions is even more pronounced. In the frog forms the width of the pre-acetabular region is equal to or only a little less than that of the post-acetabular, while in the fish forms it is only a half or two-thirds as great. In the first species the post-acetabular region has about uniform width throughout its length, while in the second it is considerably wider at the ovary than just back of the acetabulum, and becomes narrower toward the posterior end. These relations are illustrated fully in the measurements given in the table.

Braun (1900), in his diagnosis of the species of *Clinostomum* lays considerable emphasis on the structure of the anterior tip. This region is truncated obliquely ventrad so that the dorsal surface extends somewhat further forward than the ventral. Braun calls this surface the oral field. In the center of this field on the oral cone is the oral sucker. Surrounding the oral cone in both *Clinostomum attenuatum* and *Clinostomum marginatum* is the furrow and the projecting margin described by Braun in some of the species of this genus. Braun's suggestion that the whole oral field is used as a sucker has been confirmed by Osborn (1911: 368), and is fully discussed later in this paper. In *Clinostomum attenuatum* the oral cone fills a greater portion of the oral field than in *Clinostomum marginatum*, whereas in the latter the furrow is deeper and wider and the projecting margin higher. Measurements of four individuals of like contraction express the first difference. In two

specimens from the frog the oral cone measured 0.45 mm. and 0.52 mm. in diameter at its base, and the oral field had a width of 0.56 mm. and of 0.65 mm. making the ratio very nearly 4:5. But in two specimens from the fish the oral cone measured 0.60 mm. and 0.67 mm., and the oral field 0.93 mm. and 0.97 mm. in diameter, the ratio in this case being about 2:3.

Further differences between these species are found in the relation of the length of the pre-acetabular region to the total length of the body, and in the position of the genital glands. In *Clinostomum attenuatum* the region in front of the ventral sucker is short, being only about one-sixth or one-seventh the total body length, while in *Clinostomum marginatum* it is one-fourth or one-fifth. To determine the position of the genital gland field the distance from the posterior edge of the acetabulum to the ovary was measured. In the frog type the ovary is always back of the middle of the post-acetabular region, and in some cases the whole genital gland field is back of that point. In the fish type this condition is almost always reversed, the ovary in my specimens being at about the middle or in front of the middle of the post-acetabular region. Reference to the table will make these differences more clear.

In the genus *Clinostomum* the uterus empties into a sac which extends longitudinally from the genital pore antieriad toward the acetabulum (*u s* Fig. 1 and 2). In the larval stage this sac is very narrow and contracted. In both the species under consideration the uterine sac has about the same length, but its position in respect to the other organs is different. In *Clinostomum attenuatum* the distance from the posterior edge of the acetabulum to the anterior tip of the uterine sac is quite great, being almost equal to and in some cases exceeding the total length of the sac. But in *Clinostomum marginatum* the anterior tip of the uterine sac comes very close to the acetabulum and in the contracted specimens almost touches it.

Depending upon the great variation in size of the animals, the diameter of the suckers varies rather markedly in these two species. In spite of this variation distinct differences can be traced. In *Clinostomum attenuatum* the oral sucker measured on the average 0.19 mm. in transverse diameter and in *Clinostomum marginatum* 0.30 mm. The acetabulum in the first species measured on the

average 0.56 mm. and in the second species 0.73 mm. In the extreme individuals, however, the sizes overlap. The difference between these two species in the ratio of the size of the suckers is constant. In *Clinostomum attenuatum* the acetabulum is about three times as large as the oral sucker, while in the *Clinostomum marginatum* the ratio is about 2.4 to 1.

The most clear cut specific difference between these two forms is found in the difference in the structure of the cuticular spines. In *Clinostomum attenuatum* the spines range from 0.013 mm. to 0.016 mm. in length and from 0.005 mm. to 0.009 mm. in thickness at their bases, and in *Clinostomum marginatum* they vary in length from 0.007 mm. to 0.011 mm. and in thickness from 0.0015 mm. to 0.002 mm. In both species they are about equally numerous in a given area but on account of their greater width they appear more thickly set in the flukes from the frog. Figures 4 and 5 show this difference in size more clearly than any description.

The differences given above between the advanced larval stages from the frog and from the fish seem to me to be so distinct and so far reaching, that it is impossible to consider these two forms as belonging to the same species.

MacCallum (1899) and Osborn (1912) have worked out the anatomy of the adult *Clinostomum marginatum* from North America in considerable detail, so that I shall give only a sufficient description of the specimens collected from the black-crowned night heron to determine their relationship to the larval forms. The following measurements were taken from the worm shown in figure 3:

Length	3.4	mm.
Width at the anterior end	0.67	"
Width half way from the anterior end to acetabulum....	0.63	"
Width at acetabulum.....	0.56	"
Width half way from acetabulum to ovary	0.82	"
Width at ovary	1.02	"
Width half way from ovary to posterior end	0.97	"
Length of pre-acetabular region	0.97	"
Length of post-acetabular region	2.46	"
Distance from acetabulum to ovary	0.87	"
Distance from ovary to posterior end	1.4	"
Length of uterine sac	0.45	"
Distance from anterior tip of uterine sac to acetabulum...	0.07	"
Length of genital gland field	0.76	"

Transverse diameter of oral sucker	0.17	"
Transverse diameter of acetabulum	0.39	"
Ratio of oral sucker to acetabulum	1 to 2.3	"
Length of eggs (average)	0.091	"
Width of eggs (average)	0.051	"

These measurements agree so closely with those given above for the advanced larval stages from the fish that both forms must be considered as different developmental stages of the same species. This adult was very small and in length and size of suckers falls slightly below the smallest of the larval forms. The anterior end is more attenuated in the adult than in any of the larval forms which I examined, but this is due to difference in contraction. On the other hand it can be seen that the larval stage from the fish and the adult from the heron are almost exactly alike in the general shape of the body, the proportions of the various regions, the position and configuration of the genital organs, and the ratio in size of the suckers.

Osborn's description of *Clinostomum marginatum* from the bass and heron agrees in all points with my forms from similar hosts. Since he observed more individuals he notes a greater range of variation than was found in my material. His worms vary in length from 3 mm. to 8.2 mm. and in greatest width from 0.7 mm. to 2.2 mm. It is of interest to note that Osborn (1912: 191) also finds his smallest individuals among the adults, indicating a still greater variation than he has found for the larval stages.

That the fluke described by MacCallum (1899) as *Clinostomum heterostomum* is really *Clinostomum marginatum* cannot be doubted. He notes individuals up to 10 mm. in length and in his drawing of a toto preparation the ovary is back of the middle of the post-acetabular region. In all other respects his description of this worm agrees with Osborn's and my own for *Clinostomum marginatum*. These observations and comparisons seem to show that all the representatives of the genus *Clinostomum* which have been found up to date in North American fish and herons belong to the one species, *Clinostomum marginatum*.

Clinostomum marginatum was originally found in Brazil. Braun (1900) compared Rudolphi's type specimens of *Clinostomum marginatum* from Brazil with material gathered by Natterer in the same country from several different localities and hosts. He states that

all this material corresponded so closely to Rudolphi's type specimens that it must be considered as belonging to *Clinostomum marginatum*. After a careful comparison with Braun's descriptions, I can find no constant differences between the North American forms just considered and these Brazilian forms except in the greater size of the eggs of the latter. In the specimens from South America the eggs vary in length from 0.104 mm. to 0.140 mm. and in width from 0.055 mm. to 0.073 mm. The largest measurements recorded for the eggs of North American forms is 0.099 mm. in length by 0.066 mm. in width. It will be seen that in width this falls well within the range of variation of the South American flukes, and is only slightly less in length. Braun's material from Brazil agrees so exactly with Osborn's and my specimens from North America, that his figures 8, 9, or 20 (Braun 1900), might be used to illustrate our descriptions.

Clinostomum marginatum is thus a species of very wide distribution, having been reported from Brazil in South America, and from North America very widely. It has also a wide range of hosts. The advanced larval stages have been reported from cysts in the pike, the perch, the blue gill, the black bass, the rock bass, the sunfish and the trout. The adult has been found in three different genera of water birds: From three true herons, *Ardea* sp. (Brazil), *Ardea cocoi*, and *Ardea herodias*; from one species of stork, *Mycteria americana*, and from the black crowned night heron, *Nycticorax nycticorax*. The presence of *Clinostomum marginatum* in both North and South America is easily explained by the great range of of the adult host. *Ardea herodias* and *Nycticorax nycticorax naevius* for example both range over North America at large, and central and northern South America.

There remains for consideration the *Clinostomum* described by Wright (1879) from the American bittern. Wright's description and drawing of this form differs in several respects from *Clinostomum marginatum*. In his drawing the genital field is shown behind the middle of the post-acetabular region, the uterine sac reaches only about half way from the genital pore to the acetabulum, the pre-acetabular region is only about one-sixth of the total body length, and the worm is rather long and slender with fairly uniform width

throughout. All these points agree with *Clinostomum attenuatum* rather than *Clinostomum marginatum*. Since frogs form an important part of the food of the American bittern, Wright's *Clinostomum* from this host might well be the adult *Clinostomum attenuatum*.*

While collecting two adult *Clinostomum marginatum* from the heron, I was able to make some observations on the activity and relation to its host of the living parasite. The two flukes were below the average in size for this species and had only a few eggs in their uteri. The activities of the parasite were studied both while in the mouth of the heron, and after removal into normal saline solution. The heron came into my hands still alive and not more than five minutes elapsed between the killing of the bird and the finding of the flukes.

In the bird's mouth the worms were very much contracted, and adhered so firmly to the mucous membrane that it was very difficult to loosen them. Their position was well suited to resist the friction of food taken into the mouth of the heron. Not only was the acetabulum firmly attached but also the oral field functioned as a sucker. The pre-acetabular region was bent over so that the oral field was almost in contact with the acetabulum. The anterior end was given very firm attachment by the sucking action of both these structures. The post-acetabular region was much contracted longitudinally and arched so that it was quite convex. The edges were pressed closely into the mucous membrane, and evidently by the drawing up of its central part this whole region also acted as a sucker. In fact the posterior end was so firmly attached that it was almost as difficult to loosen as the anterior. Such a sucking activity of the post-acetabular region accounts for the great development of

*Since the completion of the above observations I have received from Professor Henry B. Ward material of the genus *Clinostomum* collected by A. L. Cooper from the vicinity of Go-Home Bay, Toronto, Canada. This material, which was both larval and adult, was collected from two fish hosts—*Perca flavescens* and *Micropterus dolomieu*,—one frog host—*Rana catesbiana*,—and one bird host—the American herring gull (*Larus argentatus*). A careful examination, using for species determination the points brought out above, showed that the specimens from the fish and bird belonged to the species *Clinostomum marginatum*, and the form from the bull frog to *Clinostomum attenuatum*. This gives additional data to support the hypothesis, that *Clinostomum marginatum* in its advanced larval stages is limited to fish hosts and *Clinostomum attenuatum* to frogs. Also this collection adds a new genus to the bird hosts of *Clinostomum marginatum*, and extends the list of hosts of *Clinostomum attenuatum* to two frog species.

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the dorso-ventral parenchymous muscles which has been noted for this species.

The only reference in literature to the position of the *Clinostomum* in its bird host is by Osborn (1911: 363). He found specimens of *Clinostomum marginatum* adhering to the heron's throat only by means of the anterior end. As the bird had been dead for a day or two, the worms were probably beginning to loosen their hold. In his later paper on this form Osborn (1912: 193) writes as if attachment in this species was effected by the anterior end alone. He says that the reason for the large size of the ventral sucker had not been indicated by the behavior of the worm, and that although the structure of this sucker suggests full functional power he had noticed no activities for it. My observations show that the acetabulum is not only fully functional in this species, but that it plays a very important part in holding the parasite in position in the mouth of the host.

After the position of the worms had been noted they were removed and observed for some time in normal saline solution. The living fluke was a semi-transparent whitish cream color, with the testis and ovary showing as opaque pure white areas. The intestinal ceca were dark brown, and the region surrounding the uterine sac was light pink. The animal manifested considerable activity (Figs. 6 and 7), the whole body expanding and contracting rhythmically. In its most contracted state the total length of the worm was about 2.5 mm. with a width at the region of the ovary of 1.56 mm. When most extended it reached a length of 4.96 mm. with a width at the ovary of 0.92 mm.

A study of the movements of the worm after removal from its host confirmed the observations made on its sucking activities while in position, and suggested a possible method of locomotion. This fluke has the long distance from the stomach to the mouth of the bird to travel, after the larval forms have been freed from their cysts in the fish by the digestive juices. In the most contracted position the anterior end was much shortened and turned ventrad, the oral field with the oral cone at its center being in the same plane and very close to the acetabulum. The edge of the oral field was very mobile and strong sucking movements were noted. This posi-

tion of the anterior end suggests that which was noted when the animal was attached to the mucous membrane of the heron's mouth. In the contracted position the post-acetabular region was very short and broad and so arched up as to be very thin. The edges showed considerable movement, curling in and then extending. The position and movements of this region suggested that if its edges were in contact with a soft surface considerable sucking power would be developed, just as in a small boy's leather sucker. This position of greatest contraction I shall call the sucking position. It was the assumption of this sucking position which made the removal of the worms from the mucous membrane of their host so difficult. The need of such a strong sucking reaction on the part of the parasite is apparent, when it is considered that whole fish and other very hard particles of food are taken into the mouth of the heron.

In the series of rythmical contractions made by this fluke as it lay free in the salt solution was suggested a possible method of locomotion. The cycle of expansion and contraction was as follows: From the sucking position the pre-acetabular region would stretch out and the oral field go through sucking movements. At the same time the post-acetabular region would be extended, reaching its greatest length just as the pre-acetabular region was beginning to contract. The worm would then contract again into the sucking position and a new cycle of movement would be initiated by the extension of the anterior end. Sucking movements of the acetabulum were noted during this process.

When the larval trematodes incysted in a fish eaten by the heron are liberated in its stomach by the action of the digestive juices, they turn toward the esophagus. The series of rythmical movements described above is begun, and when the pre-acetabular region is extended and turned ventrad, the oral field comes in contact with the mucous membrane. The sucking movement causes it to take hold and on the contraction of the pre-acetabular region, the worm is pulled forward until the acetabulum comes into close contact with the oral field. The acetabulum then takes hold in its turn and on the next extension of the anterior end holds the ground gained. By a laborious repetition of these movements the worm could make its way up to its final position. If any food coming

down the esophagus should strike the parasite it would contract strongly, assume the sucking position, and hold on with its whole body until the way was clear again. Having once gained its final position, the adaption of the whole body for sucking would enable it to hold its position against the friction of the heron's food.

TABLE OF MEASUREMENTS

	<i>Clinostomum attenuatum</i>					<i>Clinostomum marginatum</i>				
	1	2	3	4	5	1	2	3	4	5
Length	5.8	5.18	3.9	4.35	5.8	6.4	6.6	4.1	3.5	4.09
Width at anterior end.....	0.58	0.52	0.54	0.52	0.69	0.93	1.02	0.74	0.6	
Width half way from anterior end to acetabulum	0.84	0.71	0.61	0.61	0.86	1.2	1.3	0.89	0.78	0.96
Width at acetabulum.....	0.80	0.67	0.63	0.69	0.80	1.3	1.4	0.99	0.84	0.93
Width half way from acetabulum to ovary.....	0.84	0.67	0.61	0.60	0.93	1.5	1.54	1.17	0.89	1.17
Width at ovary.....	0.84	0.71	0.62	0.71	0.89	1.6	1.7	1.2	0.98	1.25
Width half way from ovary to posterior end.....	0.91	0.71	0.61	0.65	0.91	1.48	1.6	1.15	0.9	1.14
Length of pre-acetabular region.....	0.74	0.65	0.61	0.71	0.74	1.12	1.28	0.84	0.71	0.89
Length of post-acetabular region.....	4.5	4.41	2.73	3.18	4.5	4.1	4.43	2.55	2.34	2.91
Distance from acetabulum to ovary.....	2.33	2.42	1.67	1.86	2.18	2.08	2.05	0.99	1.01	1.38
Distance from ovary to posterior end.....	2.18	1.99	1.06	1.32	2.33	2.06	2.38	1.36	1.34	1.55
Length of uterine sac.....	0.99	1.1	0.71	0.71	0.87	1.21	1.02	0.61	0.60	0.74
Distance from anterior end uterine sac to acetabulum	0.91	0.84	0.69	0.71	1.04	0.35	0.45	0.09	0.13	0.24
Length of genital gland field.....	0.74	0.95	0.56	0.63	0.78	0.94	1.08	0.65	0.60	0.71
Transverse diameter of oral sucker.....	0.19	0.17	0.17	0.17	0.22	0.33	0.41	0.28	0.24	
Transverse diameter of acetabulum.....	0.57	0.57	0.54	0.60	0.66	0.89	0.91	0.71	0.50	

The above table gives measurements from ten representative toto mounts, five of *Clinostomum attenuatum* and five of *Clinostomum marginatum*.

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EXPLANATION OF PLATE IX

Figures 1-5 were drawn with a camera lucida.

FIG. 1. *Clinostomum attenuatum* from *Rana pipiens*. Larval specimen. X 57.

FIG 2. *Clinostomum marginatum* from *Perca flavescens*. Larval specimen. X 57.

FIG 3. *Clinostomum marginatum* from *Nycticorax nycticorax naevius*. Adult. X 57.

FIG. 4. Cuticula and spines of *Clinostomum attenuatum*. X about 1000.

FIG. 5. Cuticula and spines of *Clinostomum marginatum*. X about 1000.

FIGS. 6 and 7. Free hand drawings of a living specimen of an adult *Clinostomum marginatum* expanded and contracted. X about 33.

ABBREVIATIONS USED IN PLATE

- a*, acetabulum
- i*, intestine
- om*, oral mound
- o*, ovary
- os*, oral sucker
- t*, testis
- u*, uterus
- us*, uterine sac
- v*, vitellaria